



## **MASTERS in MEDICINE (NEUROLOGY) DISSERTATION**

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### **Motor Neuron Disease in an African population:**

*A review of current literature and a case series of the flail arm variant in the Western Cape*

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Helen Cross (HTCHEL001)

Supervisor:

Assoc Prof J Heckmann

Division of Neurology, Groote Schuur Hospital

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## **Abstract**

### *Background*

Motor neuron disease (MND) is a devastating neurodegenerative disorder, with recognised phenotypic subtypes. Although prevalent in all parts of the world, little is described in the literature with regards motor neuron disease as it occurs in African populations.

### *Aims*

This study had two main aims: to conduct a systematic review of the current available literature on motor neuron disease in persons of African genetic descent, and to describe the clinical phenotype in a subgroup of MND patients with the flail arm (FA) variant seen at Groote Schuur Hospital MND clinic.

### *Methods*

In order to identify the current published knowledge of motor neuron disease in African populations, a systematic literature review was conducted using Pubmed and Google Scholar. For the case series description, patients presenting to the Groote Schuur Hospital MND clinic with a phenotype of restricted proximal upper limb, lower motor neuron involvement for at least 12 months after symptom onset, during the time period of March 2014 to September 2016, were considered for inclusion. A full clinical description of each case, including history, examination and electrophysiological findings, was conducted.

### *Results*

Review of the available literature on MND as it occurs in persons with African ancestry revealed that little is well described. Although there are a few original studies, all are small and most are out-dated. Some trends emerged, including younger age at onset of disease, tendency to longer survival, and possibly more frequent presentation with bilateral upper limb involvement.

Six cases of FA variant of MND, representing 13% of the MND clinic cohort seen over the 2.5 years given time period, all with African genetic ancestry by self-categorization (mixed in 5, pure African in 1), are reported illustrating the various previously described features of this phenotype. Even within these few cases, there is variation in presentation and disease course.

### *Conclusions*

More research is required on African populations to address the questions surrounding MND as it occurs in Africans, including phenotypic and genetic similarities or differences to other populations. Although controversy surrounding exact case definition of the FA variant of MND remains, it does represent a unique phenotype, and seems to occur in patients of African genetic ancestry in a similar manner to that described in Caucasian populations.

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Student: **Dr Helen Margot Cross**

Student number: **HTCHEL001**

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University of Cape Town

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Supervisor:

**Assoc Prof J M Heckmann**

Division of Neurology

Department of Medicine

Groote Schuur Hospital / University of Cape Town

## Declaration

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Signature: Helen Cross

Date: 7/12/2016

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## List of abbreviations

MND	Motor neuron disease
ALS	Amyotrophic lateral sclerosis
PMA	Progressive muscular atrophy
PLS	Primary lateral sclerosis
FA	Flail arm
SNP	Single nucleotide polymorphism
TDP	Tar DNA-binding protein
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
FTD	Fronto-temporal dementia
SOD	Superoxide dismutase
FUS	Fused in sarcoma
Na	Sodium
K	Potassium
OPTN	Optineurin
VCP	Valosin-containing protein
NFKb	Nuclear factor kappa light chain enhancer of activated B cells
PCR	Polymerase chain reaction
BORG	BioOntological Relationship Graph
ID	Identity
HPO/MPO	Human/mouse phenotype ontology
SS	Sub Saharan
R	Right
L	Left
UL	Upper limb
LL	Lower limb
UMN	Upper motor neuron
LMN	Lower motor neuron
MRI	Magnetic resonance imaging
EMG	Electromyography
NCS	Nerve conduction studies
MRC	Medical research council
SMA	Spinal muscular atrophy
CIDP	Chronic inflammatory demyelinating polyradiculoneuropathy
yr	Year

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# **1. Introduction and problem identification**

Motor neuron disease (MND) is a devastating neurodegenerative disorder prevalent in all parts of the world. Aetiology is still poorly understood and is likely multifactorial. The role of genetic variants may be an important factor and is currently a much researched topic.

Most literature on the subject reports on Caucasian populations from developed countries. Little is described in the literature with regards motor neuron disease in African populations. This includes basic epidemiological data, clinical phenotypes, and the more recently topical, associated genetic findings.

South Africa has genetically diverse sub-populations, the vast majority of which have African genetic ancestry.

Motor neuron disease is not infrequently seen at Groote Schuur Hospital, one of the main referral centres in the Western Cape. A specialised motor neuron disease clinic has recently been established, providing the ideal opportunity to study this disorder in our population.

## **2. Rationale and Motivation:**

### **Background to the study with review of the literature**

#### **2.1 Motor neuron disease**

Motor neuron disease or amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of both upper and lower motor neurons, involving bulbar, cervical, thoracic and lumbosacral regions. Although there is variation in onset and progression, the disease is invariably fatal. The majority of cases are sporadic, although current reported rates of familial ALS are about 5-10% of cases (Kiernan et al., 2011).

The clinical hallmark of classic ALS is the simultaneous presence of upper and lower motor neuron signs involving the brain, brainstem and multiple spinal cord levels. Onset typically occurs in one region, with subsequent spread to other regions. Limb onset is most common (70%), followed by bulbar (25%) and then respiratory (5%) (Kiernan et al., 2011). Emotional lability and frontal lobe type cognitive impairment are also frequently part of the presentation (Kiernan et al., 2011).

MND is considered the third most common neurodegenerative disease, after Alzheimer's disease and idiopathic Parkinson's disease (Renton et al., 2014). It occurs worldwide, although the current literature does not allow for appreciation of global incidence or prevalence statistics. European population-based studies estimate the incidence to be about 2.16/100000 person-years (Kiernan et al., 2011). There appears to be a slight male predominance in sporadic ALS. In familial ALS there is equal incidence between the sexes (Kiernan et al., 2011). The peak age range in current reported literature for the onset of sporadic ALS, is 58-63 years, and 47-52 years in familial ALS (Kiernan et al., 2011).

MND remains a clinical diagnosis. There are no reliable laboratory nor imaging biomarkers. In 1994 diagnostic criteria were devised by the World Federation of Neurology working group on Motor Neuron Diseases, primarily for use in the research setting. These criteria are known as the El Escorial criteria (Brooks, 1994). They described four levels of diagnostic certainty in MND – definite, probable, possible and suspected, based on the distribution of mixed upper and lower motor neuron (U/LMN) signs within body regions (bulbar, cervical, thoracic and lumbosacral). "Definite" required UMN and LMN signs in three regions, "probable" required UMN and LMN signs in two regions, "possible" one region, whilst "suspected" was reserved for those with only LMN signs. These criteria were updated at Airlie House in 2000 and Awaji-Shima in 2008. In 2000, the "suspected" category was removed and a "laboratory-supported probable" category was added, which allowed electrophysiological evidence of LMN involvement to be considered in the probable category (Brooks et al., 2000). The 2008 Awaji criteria broadened the use of electrophysiological evidence of LMN involvement to all categories (Carvalho and Swash, 2009). (See table 1 (Al-Chalabi et al., 2016)). Despite multiple revisions these criteria still lack sensitivity and importantly, whilst defining ALS, they are not suitable for the other subtypes of MND (see below) (Al-Chalabi et al., 2016).

	Definite ALS*	Probable ALS*	Laboratory-supported probable ALS*	Possible ALS*	Suspected ALS*
El Escorial criteria (1994) <sup>14</sup>	UMN and LMN signs in three regions of the body†	UMN and LMN signs in at least two regions, with some UMN signs rostral to LMN signs	..	UMN and LMN signs in only one region, or UMN signs alone in two or more regions, or LMN signs rostral to UMN signs	LMN signs only
Airlie House criteria (2000) <sup>15</sup>	UMN and LMN signs in the bulbar region and at least two spinal regions, or UMN signs in at least two spinal regions and LMN signs in three spinal regions	UMN and LMN signs in at least two regions, with some UMN signs rostral to LMN signs	Clinical evidence of UMN and LMN signs in only one region, or UMN signs alone in one region and electrophysiological evidence of LMN signs in at least two regions	UMN and LMN signs in only one region, or UMN signs alone in two or more regions, or LMN signs rostral to UMN signs	..
Awaji-Shima criteria (2008) <sup>16</sup>	Clinical or electrophysiological evidence of UMN and LMN signs in the bulbar region and at least two spinal regions, or UMN and LMN signs in three spinal regions	Clinical or electrophysiological evidence of UMN and LMN signs in at least two regions, with some UMN signs rostral to LMN signs	..	Clinical or electrophysiological evidence of UMN and LMN signs in only one region, or UMN signs alone in two or more regions, or LMN signs rostral to UMN signs	..
LMN=lower motor neuron. UMN=upper motor neuron. ..=components are not part of the classification. *Neuroimaging and clinical laboratory studies must be done to exclude alternative diagnoses. †Regions: bulbar, cervical (corresponding to neck, arm, hand, diaphragm, and cervical spinal cord-innervated muscles), thoracic (corresponding to back and abdomen muscles), and lumbar (corresponding to back, abdomen, leg, foot, and lumbosacral spinal cord-innervated muscles).					
<b>Table 1:</b> The El Escorial criteria and its revisions					

**Table 1: The El Escorial criteria and its revisions, taken from (Al-Chalabi et al., 2016)**

## 2.2 Disease variants

Distinct disease phenotypes within MND are recognised. The first to be described and probably still the best characterised are the classic limb onset ALS, progressive bulbar palsy and progressive muscular atrophy phenotypes.

In classic limb onset ALS, symptoms are first noted by the patient in the upper or lower limbs (often with more widespread signs than symptoms) followed fairly rapidly by involvement of other segments such as bulbar or respiratory muscles. Progressive bulbar palsy, as the name suggests, starts with prominent weakness of the bulbar musculature before spreading to other regions. This phenotype has consistently been linked with a poorer prognosis and appears to be more common in women (Kiernan et al., 2011). In progressive muscular atrophy (PMA), there is exclusive lower motor neuron involvement. This subtype of MND typically has a longer survival. Primary lateral sclerosis (PLS) is a purely upper motor neuron variant of MND, but is extremely rare. Definitive diagnosis of this variant should be delayed at least 4 years, as lower motor neuron features could develop during this time (Gordon et al., 2006).

Two further MND variants were more recently included: flail arm (FA) and flail leg variant. These represent lower motor neuron disorders of the upper and lower limb respectively. In the FA variant weakness and wasting occur proximally and fairly symmetrically in the upper limbs. In contrast, the flail leg variant is characterised by predominant distal involvement in the lower extremities.

A final phenotypic variant to be mentioned is Mill's variant (also known as hemiplegic ALS), in which there is a predominant upper motor neuron, hemiplegic pattern of involvement. It is controversial whether this is a true subtype or merely a descriptive clinical pattern of PLS disease onset (Ravits et al., 2013).

The flail arm variant was first described by Vulpian in 1886 and Gowers in 1888 and has been recognised under several names: Vulpian-Bernhardt Syndrome, brachial amyotrophic diplegia, hanging-arm syndrome, dangling arm syndrome or neurogenic man-in-the-barrel syndrome. The

variant accounted for about 10% of MND patients at one referral centre (Hu et al., 1998). Given the prominent, symmetrical involvement of the proximal arm muscles with sparing of the legs and bulbar muscles it is an easily clinically recognisable phenotype. There may be milder distal upper limb weakness (in contrast to the more severe distal weakness and wasting of limb onset ALS). It has been shown to have more of a male predominance than other MND variants (Wijesekera et al., 2009, Hu et al., 1998).

Bilateral arm weakness may be the presenting feature in classic limb onset ALS in 5-10% of such cases, yet in FA variant (see definition below) the pattern of weakness remains more restricted over time (Katz et al., 1999). Flail arm variant has been shown to differ from upper limb onset ALS in terms of distribution and severity of weakness and wasting at presentation (Yoon et al., 2014). While ALS presents with prominent distal arm weakness and the FA variant with proximal weakness, both show similar age at onset and electrophysiological findings (Yoon et al., 2014). Additionally, studies have shown pathology typical of ALS in the flail arm subgroup (Sasaki, 2007), although predominantly restricted to the cervical region.

There are two schools of thought with regards the exact definition of this phenotype. Wijesekera et al describe a more widely inclusive flail arm variant in which some cases have pathological deep tendon reflexes and whilst remaining restricted to the upper limbs for 12 months post onset of symptoms, many patients (22-77%) will eventually develop signs in the lower limbs and bulbar regions (Wijesekera et al., 2009). They argue that the FA phenotype is associated with a significantly improved survival, which appears to be linked to time to spread to the second region of involvement (Wijesekera et al., 2009).

Katz et al (Katz et al., 1999) are more restrictive in their definition and label the syndrome brachial amyotrophic diplegia. They describe a case series of 10 such patients by including those with lower motor neuron signs confined to the arms for more than 18 months. Seven of these patients had a relatively stable course after an initial rapid deterioration, and failed to develop signs outside of the upper limbs after a mean follow-up of 67 months. Nine out of ten were completely pure lower motor neuron syndromes. On the basis of their findings, they argue that there may be a subgroup even within the FA variant which has greatly improved survival. They propose that this group could perhaps rather be considered a variant of PMA than of ALS as a whole.

## **2.3 Aetiology and neuropathology**

The pathophysiological mechanisms underlying motor neuron disease are not yet fully elucidated. It seems most likely to be due to multifactorial processes with complex interactions between genetic and molecular pathways (Kiernan et al., 2011).

What is clear, is that there is loss of upper and lower motor neurons, with axonal degeneration along their projection course from the motor cortex to the lateral columns ("lateral sclerosis") and from the anterior horn cells to the peripheral nerves, leading to denervation of muscles ("amyotrophic"). There are also changes in the neuronal support cells (astrogliosis, spongiosis and microglial activation), but whether these changes are part of the pathological process or merely secondary is still unclear (Ravits et al., 2013).

In 1988 Leigh et al and Lowe et al (Leigh et al., 1988, Lowe et al., 1988) identified the deposition of ubiquitin in the cytoplasm of ALS motor neurons. Ubiquitin is known to have a maintenance-type role within the cell, in the realm of protein homeostasis. It was suggested that perhaps a pathological protein was being identified for removal by the cell. Similar changes were also noted in brains of patients with frontotemporal dementia. This pathological protein was identified as TDP-43 in 2006 (Neumann et al., 2006, Arai et al., 2006). TDP-43 is a nuclear protein involved in RNA and DNA processing, which in ALS and FTD was being translocated to the cytoplasm and altered into a pathological form (Ravits et al., 2013). In the majority of ALS, these ubiquitinated inclusions of TDP-43 are the hallmark of the neuropathology. Other proteins with a role in ALS neuropathology have subsequently been discovered, largely in connection with genetic discoveries (see below.) Four main neuropathological subtypes are now recognised (Ravits et al., 2013). Firstly TDP-43 proteinopathy described above (in the majority of patients). Secondly, C9ORF72 related TDP-43 proteinopathy, which is similar but also has deposits of a secondary pathological protein, p62. The third type is the deposition of ubiquitinated SOD1 protein. In this group, the pathological burden is greater in the lower rather than the upper motor neurons. Finally a FUS proteinopathy has basophilic inclusions which contain FUS protein (Ravits et al., 2013).

The different neuropathological findings are linked to some extent to the underlying genetic abnormality, where identified. The pathology does not seem to vary between the different clinical phenotypes, rather there is a differing distribution of disease load, accounting for the phenotypic differences (Ravits et al., 2013). That said, the relationship of the phenotype, neuropathology and genetics are not yet fully understood.

There appears to be a trigger initiating signalling pathways leading to neurodegeneration, then propagation of the pathophysiological process neuroanatomically, and finally neuronal death. It has been suggested that the propagation occurs in a prion-like fashion, due to the actions of the abnormal proteins. There may also be a role for the neuronal support cells (astrocytes, oligodendrocytes and microglia) in terms of handling of toxic substances or non-provision of appropriate trophic support.

Pathophysiological processes implicated in MND include glutamate-induced excitotoxicity, oxidative stress with the generation of free radicals, disruption of axonal transport systems, mitochondrial abnormalities, Na<sup>+</sup>/K<sup>+</sup> pump dysfunction, and as suggested above, insufficient neurotrophic factors, excessive neurotoxic compounds, glial cell dysfunction and cytoplasmic protein aggregations interfering with RNA and DNA metabolism (Kiernan et al., 2011). All of these mechanisms could induce signs of MND but none are able to cause pathology in the scale required for the severity of disease when activated alone. This implies a multifactorial process, and that most likely, these are not the primary causative factors (Musrò, 2013).

There have been some suggested environmental risk factors which may impact on the above processes, possibly with a triggering role. Intensive physical exertion, including professional sport and armed service, have been linked to an increased rate of ALS (Harwood et al., 2009, Kasarskis et al., 2009, Chiò et al., 2005). Other proposed environmental risk factors include cigarette smoking (Gallo et al., 2009), low or high maternal age and “exposure to younger siblings” (Fang et al., 2008).

Certain neurotoxins may also play a role as was found in a cohort of MND patients in Guam (Cox and Sacks, 2002). A particular neurotoxin suspected to contribute to the development of MND in susceptible individuals is beta N methylamino L alanine (BMAA) produced by marine cyanobacteria found in blue green algae (Pablo et al., 2009).

## **2.4 Genetics of motor neuron disease: summary of current knowledge**

The first gene associated with ALS was superoxide dismutase 1 (*SOD1*) in 1993 by Rosen et al (Rosen et al., 1993). *SOD1* has been shown to be associated with about 12% of familial cases and about 1% of sporadic cases. There is considerable phenotypic heterogeneity amongst these mutation carriers.

The next ALS gene discovery was not until 2008 when TAR DNA-binding protein (*TARDBP*) mutations were found (Sreedharan et al., 2008). This followed on the discovery of the TDP-43 protein, which is a major component of the ubiquitin-positive neuronal inclusions which are now seen as a pathological hallmark of ALS and fronto-temporal dementia (FTD) (Neumann et al., 2006). The mutation is known to cause about 4% of familial ALS. The characteristic pathology is however much more widespread (Renton et al., 2014).

Missense mutations in fused in sarcoma (*FUS*) gene on chromosome 16 were discovered shortly after TARDBP (Kwiatkowski et al., 2009). This mutation causes similar pathology to TDP-43, but with FUS inclusions. A similar gene, *Ubiquilin2*, which was also linked to ALS, encodes the protein which regulates proteasome degradation of ubiquitin proteins (Deng et al., 2011). Both these proteins are suggested to have a role in the development of protein aggregate inclusions and aberrant RNA processing.

Mutations in optineurin (*OPTN*) were initially described in Japanese familial ALS (Maruyama et al., 2010) and have remained most common in this population group. This gene has also been implicated in the seemingly unrelated disorders of primary open angle glaucoma and Paget's disease of bone (Renton et al., 2014). *OPTN* regulates many different cellular processes, which may relate to its phenotypic variability with mutations. What it perhaps highlights is the concept of ALS as a consequence of tissue-specific expression of possibly several interacting pathways. This idea of a multisystem proteinopathy was raised when valosin-containing protein (*VCP*) gene mutations were found to cause ALS as well as the syndrome of inclusion body myopathy with Paget's disease and frontotemporal dementia (IBMPFD) (Kim et al., 2013, Johnson et al., 2010).

Sequestosome 1 (*SQSTM1*) mutations are also linked to ALS (Fecto et al., 2011) and Paget's disease. This gene encodes a protein (p62) that regulates ubiquitin binding and NFκB signalling.

Hexanucleotide repeat expansions in *C9orf72* on chromosome 9 (Renton et al., 2011) were discovered to cause ALS in 2011. This was an important milestone, as this gene has been shown to be the cause of about 40% of familial ALS cases in people of European ancestry (Majounie et al., 2012).

Mutations in profilin 1 (*PFN1*) are not a common cause of familial ALS, but were an important discovery in that they highlighted a new pathogenic pathway, namely disruption of the neuronal cytoskeleton (Wu et al., 2012).

The above mentioned nine genes are the current most established genes linked to ALS. Mutations in several other genes (>20 in total) have been raised, but the genetic evidence for a link with ALS is weaker (Renton et al., 2014).

With the development of next generation sequencing, it has become possible to examine multiple genes associated with ALS in parallel, in a relatively unbiased approach. It has been found that some ALS patients carry pathogenic variants in more than one known ALS gene (van Blitterswijk et al., 2012). Cady and colleagues (Cady et al., 2015) hypothesised that some of the apparently sporadic cases of ALS may be due to the co-occurrence of two or more genetic variants with synergistic negative effects. This group analysed 17 ALS-associated genes in 391 ALS patients from a US database, 10% of which had a family history of ALS; 4% of study participants had variants in more than one gene (14% of familial cases, 3% of sporadic cases), supporting their hypothesis. In addition to this, novel or rare coding variants were discovered in 64% of the familial and 28% of the sporadic cases, highlighting the utility of the current next generation sequencing in furthering our knowledge of the genetics of ALS.

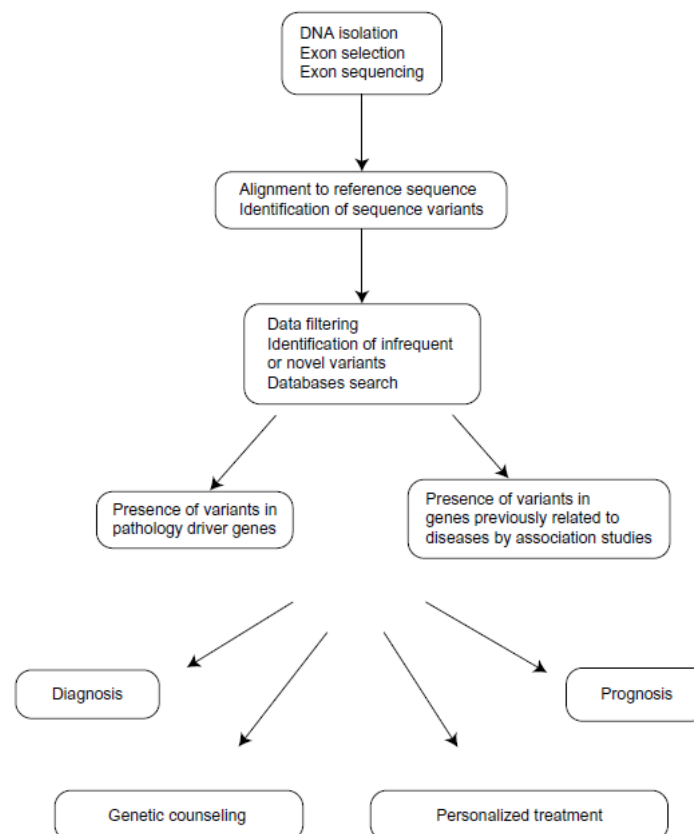
Although the focus of this dissertation is on the clinical characterization of MND patients with predominant lower motor neuron involvement in the arms, seen at our MND clinic between June 2014 and September 2016, we also have an opportunity to collaborate with a bioinformatics team to use whole exome sequencing (WES) to discover genetic variants in one of these cases and his unaffected parents (family trio). The results of the WES will not form part of this dissertation, but I will discuss the principles of WES and gene variant discovery as this is potentially a powerful tool to discover the molecular underpinning of neurological diseases and will become increasingly available in the clinic.

## **2.5 Whole exome sequencing**

Whole exome sequencing (WES) is an application of the next generation sequencing technology that involves examining only the protein coding genes, or exome, from the greater genome. The exome consists of about 200,000 exons (Ku et al., 2012), which constitutes about 1% of the total human genome (Teer and Mullikin, 2010). Exome sequencing can therefore only detect mutations in these regions of genes. Although WES has been a successful strategy to identify pathogenic variants in monogenic inherited disorders due to truncated or missing protein products, usually in individuals with a clear family history such as autosomal dominant or recessive muscular dystrophy, its usefulness in sporadic disorders is less clear. The aim of this approach is to identify pathogenic variation, both rare causal variants and risk variants, which may be responsible for disease, whilst being cheaper than approaches such as whole genome sequencing (Singleton, 2011).

The principle of WES is that a genomic DNA sample is first sheared into small fragments of about 200bp and then hybridized to prefabricated DNA probes (the capture kit). This helps to separate out the exons. The hybridized exons are then captured by antibody marked beads, purified and

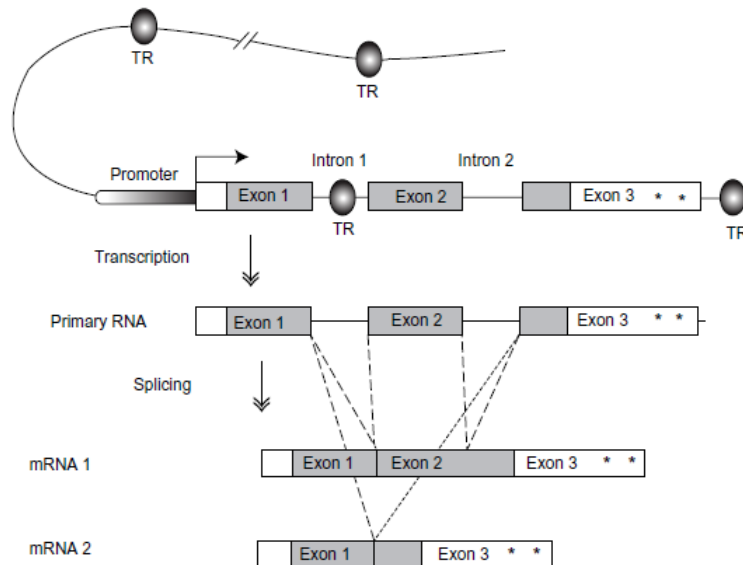
amplified using PCR via high throughput parallel sequencing. Once sequenced, the genomic data is mapped against a reference human genome to identify deviations. Polymorphisms that are found in control human databases or predicted to be non pathogenic are excluded. The remaining rare variations are then subjected to bioinformatic tools which can predict whether the sequence variations are likely to be non-synonymous (result in a change in produced protein) or cause loss of protein translation. These would then become potential disease candidates (Singleton, 2011, Glass and Nuara, 2013, Narayanaswami, 2015).



**Figure 1: The process of whole exome sequencing, taken from (Sastre, 2014)**

Whilst excellent for identifying genes for highly penetrant Mendelian disorders and single nucleotide variations, whole exome sequencing may miss variations in non-transcribed regions of the genome (such as introns, promoters and regulatory elements), variations that alter mRNA splicing, variations regulated epigenetically and variations in the forms of deletions, duplications (including sequence repeats) and translocations (Sastre, 2014, Glass and Nuara, 2013). For these possibilities, it would be best to utilise alternative methodologies such as whole genome sequencing (Glass and Nuara, 2013).





**Figure 1** Schematic representation of gene structure and expression.  
**Notes:** Protein-coding genes are composed of exons that contain protein-coding information (boxes), separated by noncoding introns (lines). Gray boxes indicate exon protein-coding regions and white boxes represent mRNA 5' and 3' un-translated regions. Genes are transcribed from the promoter regions, immediately upstream of exon 1. The transcription start site is indicated by an arrow. Gene expression is controlled by a number of TR regions that can be located upstream or downstream of the gene, at variable distances, or inside the gene (most frequently in introns). The mRNA stability and translation can be regulated by microRNA binding to specific sites in the 3' un-translated region (indicated by asterisks). Genes are transcribed into primary RNAs containing introns and exons. Subsequent splicing processes eliminate the introns to generate mature mRNAs. Alternative splicing processes can give rise to different mRNAs, depending on the exons that they contain (mRNA1, mRNA2), that code for different protein isoforms.  
**Abbreviations:** TR, transcription regulatory region; RNA, ribonucleic acid; mRNA, messenger ribonucleic acid.

**Figure 2: Schematic representation of gene structure and expression (Sastre, 2014)**

The above figure, taken from (Sastre, 2014), highlights the elements of gene structure and the process of transcription. This makes it easy to see that when only examining the exome, important regulatory regions are not examined. This could have implication for missing causative gene mutations particularly in complex diseases where altered gene dosage may be critical (Nel, 2016).

Exome sequencing is helpful in identifying variants in known disease-causing genes, as well as identifying genes not previously known to cause disease (Singleton, 2011). Additionally it is likely to be beneficial in finding mutations in genes known to cause disease, but which are phenotypically different to that currently being examined. For example, the discovery of the VCP mutation causing ALS (Johnson et al., 2010). This gene was previously linked to Paget's disease of the bone and inclusion body myopathy.

### **3. Research Aims and Objectives**

#### **3.1 Aims of the study**

There were two main aims of this study:

1. To conduct a systematic review of the current available literature on motor neuron disease in persons of African genetic descent.
2. To describe the clinical phenotype in a subgroup of MND patients with the flail arm variant seen at Groote Schuur Hospital MND clinic between March 2014 and September 2016.

An exploratory aim was to lay the foundations for a genetic sub-study:

To initiate descriptive disease terms, to be used to generate ontological terms, to be used to construct an MND-semantic graph for bioinformatic data mining in a subsequent sub-study of an MND flail arm trio.

#### **3.2 Hypotheses**

1. Current literature on motor neuron disease in African populations is limited, especially for the sub-Saharan region.
2. Flail arm variant patients of African ancestry seen at Groote Schuur Hospital, have similar phenotypic presentations to those described in Caucasian populations.

## 4. Research Design and Methods

A dedicated motor neuron disease clinic was established at Groote Schuur Hospital Division of Neurology in March 2014 to better provide for the multidisciplinary needs of the hospital's MND patients. This clinic also provides a perfect research opportunity to study this population. All patients attending the clinic are approached about being involved in African research into MND. Where informed consent is obtained, clinical data is captured and blood samples taken for storage for potential genetic analysis. This then forms part of the MND clinical database and DNA repository. Within this setting, the current study has taken place.

There are three components to this study:

### 1. Systematic review

In order to identify the current published knowledge of motor neuron disease in African populations, a systematic literature review was conducted using Pubmed and Google Scholar. The following key words in various combinations were used: *amyotrophic lateral sclerosis/ALS; motor neuron disease/MND; MND/ALS variants; flail arm variant; brachial amyotrophic diplegia; Vulpian-Bernhardt syndrome; phenotype; genotype; genetics; exome sequencing; African; Sub Saharan Africa; African-American; race; ethnicity; differences*. Additional studies were identified through the reference lists provided by studies found in the above manner.

### 2. Clinical phenotype description

Patients presenting to the Groote Schuur Hospital MND clinic with an upper limb, lower motor neuron presentation of motor neuron disease, with initial sparing of lower limbs and bulbar musculature, during the time period of March 2014 and September 2016, were considered for inclusion in the case series. Eligible patients for the FA variant study had symptoms confined to the upper limbs (predominantly proximal) for at least 12 months prior to spread to other regions. A full clinical description of each case, including history, examination and electrophysiological findings, was conducted.

### 3. Preparation for genetic sub-study

We have the opportunity to participate in a WES study in collaboration with colleagues at the South African National Bioinformatics Institute, University of the Western Cape, with the aim of detecting novel disease-associated genes using a trio-based approach. Family analysis of case-unaffected parent trios is a powerful way to investigate for recessively inherited (homozygous/compound heterozygous) or de novo mutations. Both of these mechanisms could give rise to an apparently sporadic case of ALS with actual genetic causation (Steinberg et al., 2015).

Two patients from the above case series with the distinct flail arm phenotype of MND were consented to specifically participate in a genetic sub-study. One family trio (affected individual with both unaffected living parents) were invited to participate and an additional case without living

parents. The parents' DNA was to provide useful control data. Whole exome sequencing will be performed on these four participants in an attempt to identify protein coding variants associated with flail-arm variant of MND in this population.

To assist our bioinformatics collaborators in the development of a specific BioOntological Relationship Graph (BORG) for MND, Dr Helen Cross created a list of clinical terms describing phenotypic, anatomic, pathological and genetic features associated with MND (see table 2). These terms were converted to official ontology terms and identities (HPO/MPO - Human/mouse phenotype ontology) by the genomicists in order to create the disease model in BORG. This database is used to assess the likely biological impact of genetic variants. It employs a concept of cross-ontology linking. Multiple sources of genomic and biomedical knowledge (phenotypes and disease pathways) are combined into a semantic network, using standard ontological terms. This allows indirect associations to be uncovered via biologically plausible links (Saunders et al., 2016, Dashti et al., 2012).

Once identified in the above manner, the candidate variants will be systematically interrogated by the clinicians (Dr Helen Cross and Prof Jeannine Heckmann) to exclude potentially false positive variants based on what is known about the MND. This sub-study is however still ongoing, and the results do not form part of this thesis.

Table 2: MND semantic terms to inform gene ontology BORG terms				
Clinical terms	Signs & Symptoms	Pathophysiology	Anatomy	Genes
Amyotrophic lateral sclerosis Motor neuron disease Flail arm variant Brachial amyotrophic diplegia Vulpian-Bernhardt syndrome Hanging arm syndrome Neurogenic man-in-a-barrel syndrome Split hand syndrome	Wasting Weakness Cramps Fasciculations Atrophy Flaccid Hypotonia Hypertonia Areflexic Spasms Dysarthria Dysphagia Dyspnoea	Neuronal degeneration Axonal degeneration Oxidative stress Glutamate excitotoxicity Mitochondrial dysfunction Defects in axonal transport Defects in glial cell function Microglial activation RNA processing defects Neuronal protein aggregates (ubiquitin/TDP43/FUS) DNA/RNA binding proteins abnormalities Caspase activation Inactivation of excitatory amino acid transporter 2 Nerve hyperexcitability	Lower motor neuron Upper motor neuron Axon Denervation Anterior horn cell Frontal lobe Motor cortex Corticospinal tracts Motor unit Muscle	TARDBP (TAR DNA binding protein) ( <i>associated with upper limb onset</i> ) FUS (fused in sarcoma) ( <i>associated with LMN predominance</i> ) SOD1 (superoxide dismutase 1) ( <i>associated with LMN predominance</i> ) C9Orf72 ALS2 (amyotrophic lateral sclerosis 2) ANG (angiogenin) DAO (D-amino-acid oxidase) FIG4 NEFH (neurofilament, heavy polypeptide 200kDa, heavy chain) OPTN (optineurin) SETX (senataxin) VAPB (vesicle associated membrane protein B and C) VCP (valosin containing protein) DCTN1 (dynactin 1) PFN1 UBQLN2 SQSTM1 TAF15 EWSR1 VEGF SMN1

## **5. Ethics**

### **5.1 Ethical considerations**

Informed consent was taken from each participant in the study, for the inclusion of clinical data as well as for genetic analysis. Informed consent covered public sharing of genetic information should a variant be discovered.

All patients received information on MND and whole exome sequencing, in their first language.

Participants' identity was kept confidential throughout the study, through the use of an assigned study identification code. The original consent documents and data capture sheets containing the patients' names were kept in a locked office in the Division of Neurology, Groote Schuur Hospital.

### **5.2 Ethics approval**

The University of Cape Town Human Research Ethics committee approved this study for a Masters in Medicine degree: HREC (577/2015).

## **6. Results and discussion**

### **6.1 Systematic review**

#### **Motor neuron disease in people of African descent: what is known?**

##### **Background**

African genetic ancestry is ancient and diverse. Africa is thought to be the ancestral homeland of all modern humans according to evolutionary studies (Tishkoff and Williams, 2002). More recently, one of the largest migrations in human history was that of Africans (predominantly from West Africa) to Europe and the Americas as part of the slave trade, which resulted in African genes being transported to these areas and admixed with local indigenous genes, as well as those of other transcontinental migrants. African genetics and the possible role they play in complex diseases are therefore important areas for further research with more wide reaching consequences than may initially be considered. Additionally, as we understand more about the causation of disease, the role of environmental influence (possibly interacting with genetic susceptibility) becomes more important. Due to different socioeconomic and environmental factors, the environment in which most Africans live is vastly different to most individuals from first world Western countries. This could lead to epigenetic influences on genetic traits. For these reasons, the study of disease as it occurs in African populations has importance for us all.

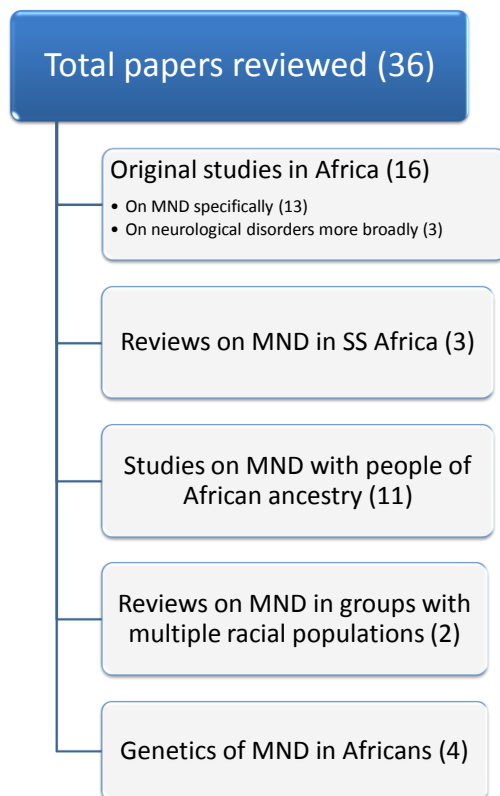
For those working in Africa, it is also critical to understand if our patients differ from those in other regions of the world in terms of phenotypic expression of disease. In all diseases, but especially those with a significant genetic contribution, the consideration of differing genetic ancestry in different study populations becomes important. The only way to study this fully is to collect data from our setting and compare it to that found in other populations. An important starting point is to consider what is already reported in the published literature. To this end, a systemic review of knowledge regarding motor neuron disease in people of African descent was undertaken.

##### **Review of the literature**

In order to identify studies on MND in African populations, a systematic search was conducted as outlined in the methods section.

This search identified 36 research papers in total: 16 studies were original research from African countries, 11 studies were original research on mixed populations, not in Africa, but containing persons with African genetic ancestry, three papers were reviews or commentaries on MND in African populations, two papers were reviews on MND in groups with multiple different ethnic/racial populations, and four articles made mention of MND genetics in African people (See figure 3). Studies written in French were excluded, except for two where an English abstract was available. Mention was made in the reference lists of the above studies of six other reports on MND in Africa. These references could not be traced; however four out of six appeared to be in French. Studies reporting on spinal muscular atrophy and konzo were not considered. Although these disorders are

also a type of motor neuron pathology, they are not considered to be classic motor neuron disease and did not therefore form part of this study.



**Figure 3: Papers reviewed for African MND systemic review**  
(MND = motor neuron disease; SS = sub-Saharan)

## Findings

### *Studies conducted on MND in Africa*

Papers reporting on 16 studies conducted on MND in Africa were analysed. 13 focussed specifically on MND, whereas three were on neurological or neurodegenerative disorders more broadly. The time span of publication was wide, and the majority of the papers (10) were published prior to 2000.

The three reviews on MND in sub-Saharan Africa (Lekoubou et al., 2014, Marin et al., 2012, Quansah and Karikari, 2015) largely represented summaries of the above original papers and were used primarily to find these studies. They were not analysed further.



<b>Table 3: African motor neuron disease studies</b>							
<b><i>Motor neuron disease specific</i></b>				<b><i>Neurological/Neurodegenerative disorders non specific</i></b>			
<b>Study</b>	<b>Country</b>	<b>Number of patients</b>	<b>Type of study</b>	<b>Study</b>	<b>Country</b>	<b>Number of patients</b>	<b>Type of study</b>
(Abdulla et al., 1997)	Sudan	28	C	(Kengne et al., 2006)	Cameroon	10	C and E
(Adam, 1992)	Kenya	47	C	(Osuntokun et al., 1987)	Nigeria	NA	E
(Brah et al., 2014)*	Togo	5	C	(Tekle-Haimanot et al., 1990)	Ethiopia	NA	E
(Cosnett et al., 1989)	South Africa	86	C				
(Daude, 2014)	South Africa	48	C				
(Dean and Elian, 1993)#	South Africa	NA	E				
(Eze and Kalu, 2014)#	Nigeria	1	E				
(Harries, 1956)	Kenya	2	C				
(Imam and Gunniyi, 2004)	Nigeria	16	C				
(Osuntokun et al., 1974)	Nigeria	92	C				
(Radhakrishnan et al., 1986)	Libya	23	C				
(Sene et al., 2003)*	Senegal	33	E				
(Wall and Gelfand, 1972)	Zimbabwe	13	C				

\*Only abstract available – full paper in French and not accessible

# Not useful studies – information too limited

E= Studies rendering purely epidemiological type data such as rates and prevalences

C= Studies rendering clinical information about disease presentation

NA=not applicable (epidemiological studies looking at extrapolated rates of disease in a population)

The above table summarises the African MND studies reviewed. The studies by Eze and Kalu and Dean and Elian will not be considered further. The paper by Dean examined relative rates of mortality due to MND in white South Africans, comparing those who were immigrant from Europe and those who were South African born (Dean and Elian, 1993). The paper by Eze looked at neurological admissions to a teaching hospital in Nigeria over a 1 year period. Only 1 case of MND was included, with no clinical information (Eze and Kalu, 2014).

The first clinical report on MND in Africans described 2 cases seen in Kenya in 1954. Both cases were young (26 and 30 years), and both male, but otherwise had typical mixed upper and lower motor neuron signs involving the cranial, cervical and lumbosacral regions, with sparing of sensation and sphincter function (Harries, 1956).

Two reports were published in the 1970s. In 1972 Wall described 13 cases from Rhodesia (now Zimbabwe). His report (based in the Department of Medicine, University Hospital) also indicated a young age of onset, mean 36 years, and a male predominance (10:3). He found no geographical clustering or familial cases. Typical mixed upper and lower motor neuron signs, with absence of sphincter involvement were described. One unusual feature he noted was sensory symptoms without signs in 6 of his patients. Of note, his patients had no spinal imaging or electrophysiological testing done (Wall and Gelfand, 1972).

Osuntokun's 1974 study from Nigeria, was the first prospective study on MND in an African population. It described all cases of ALS (73), progressive muscular atrophy (10) and chronic spinal muscular atrophy (9) diagnosed and followed up at University College Hospital, Ibadan, between 1966 and 1973. Diagnoses were based on clinical, electrophysiological, imaging, blood, cerebrospinal fluid and muscle biopsy information. He described the three categories of motor neuron diseases separately. The ALS group had a mean onset of 39 years of age, with 23% younger than 30 years. The male to female ratio was 3:1. No significant familial cases, but four patients had had previous polio. Clinical presentation was typical for MND in the limbs, with 40/73 having additional prominent bulbar involvement. Four cases also had parkinsonian features. Survival was relatively long: 54% >6 years, 29% >10 years and 8% >15 years. Osuntokun discussed possible reasons for this earlier age of onset with longer survival in his population, considering the role of the polio virus, dietary toxins, possible chronic organophosphate or lead poisoning and trauma. The progressive muscular atrophy group of ten patients had a mean age of onset of 37.8 years, and 6/10 patients survived longer than six years. The information on chronic spinal muscular atrophy will not be considered further (Osuntokun et al., 1974).

Radhakrishnan et al also conducted a prospective study on motor neuron disease in Libya, published 1986. He describes the Libyan population to be very mixed given its Mediterranean location: "less than 1% of the Benghazi population is pure black". Radhakrishnan captured all cases from North Eastern Libya diagnosed with MND in a five year period. Diagnoses were based on clinical, electrophysiological, imaging and laboratory data; 23 cases included: 17 ALS, four progressive muscular atrophy and two progressive bulbar palsy. Mean age of onset was 47.4 years. Median survival was 42 months. There were no familial cases. Population data from the 1984 Libyan census was used to calculate an average incidence rate of MND at 0.89/100000 population/year (Radhakrishnan et al., 1986).

The first South African MND study was that of Cosnett et al from 1989, which retrospectively analysed 86 patients with MND across various racial groups, including 59 black African patients. Across all the racial groups there was a male predominance – 3:2 in the black patients. Some interesting features included earlier mean age of onset amongst the black group (47.4 years versus 54 years in the white and Indian groups) and a relatively greater proportion of bulbar palsy and progressive muscular atrophy amongst females, especially in the black group. 56% of the black patients with MND came from rural areas, which was higher than that for the average hospital population. The authors speculated whether this could have a causative link, possibly environmental exposures or increased injury rates (Cosnett et al., 1989).

Adam described 47 cases of MND over a 10 year period in Nairobi, Kenya, published 1992, with half of the cases collected prospectively. Again there was a male predominance (2.8:1), and all three major types of MND were described (ALS, progressive muscular atrophy and progressive bulbar palsy). Although a small study, they observed a bimodal age distribution, with peaks in the fourth and sixth decades of life. The younger group (30-39 years at onset) was unusual in that the patients experienced a rapid course of disease with 7/9 dead within a year of diagnosis (and the other two lost to follow up) (Adam, 1992).

The first MND study from Sudan was published in 1997 by Abdulla et al. He described 28 patients diagnosed with MND at teaching hospitals over an 18 month period. Age range at onset was 13-84 years, mean 40 years. If considered by ALS category, the progressive muscular atrophy group had the youngest age of onset at 26.5 years. This was the first African study to describe familial cases. There were four cases with family history of MND. These cases had even younger onset (mean age 20.7 years) and they also all had sensorineural hearing loss (as did one of the sporadic cases). Overall, the commonest presentation of MND was with initial bilateral upper limb involvement (39%), followed by bulbar onset (21%). None of the patients had dementia or parkinsonism. Survival data is not included for all the patients, but three survived longer than eight years (Abdulla et al., 1997).

Of the four studies post year 2000, three gave only limited information. Sene et al from Dakar 2003, wrote a retrospective review of the cases of MND at the University Hospital over eight years. Only the abstract was accessible, but it stated 33 cases were collected, with an age range of 16-77 years (Sene et al., 2003). Imam et al provided an “update” on MND in Nigeria: 16 further cases over a 20 year period were described retrospectively, confirming the earlier reports of male predominance (here 15:1) and young age of onset (here 38.6 years mean). Trauma was reported as the most frequent risk factor (Imam and Gunniyi, 2004). Brah et al reported on MND cases from Lome in Togo. Apparently only 5 cases satisfying El Escorial criteria for definite ALS were diagnosed during a 10 year period out of 10 128 patients seen at the neurology department. The average age at onset was 49 years, and the average duration of disease was 17.6 months. All cases were male (Brah et al., 2014).

The latest series on MND in Africans comes from South Africa. Daude and Combrinck described the results of a folder review of 48 patients from a Cape Town teaching hospital over a five year period. This included 43 sporadic cases and five familial. The male to female ratio was 3:2. Different racial groups were represented: eight white patients, 11 black African patients and 29 mixed African genetic ancestry patients. The mean age of onset tended to be higher amongst the whites/Caucasians (58.8 years) versus black African population (45.9 years). There appeared to be a disproportionate number of MND patients with a work history in the chemical or agricultural industries (19%) compared to estimated 8% exposed to chemicals in the overall workforce in Western Cape region (Daude, 2014).

The three broader studies provided only epidemiological type data. Kengne et al audited case notes of degenerative brain disorders as a proportion of general neurological case consultations over a nine year period at the two main teaching hospitals in Yaounde, Cameroon. Degenerative brain disorders accounted for 4% of the total consultations, with ALS accounting for 12% of this subgroup. Of the ten ALS patients included, eight male and two female, the mean age at presentation was 50.9

years (Kengne et al., 2006). Osuntokun's group from Nigeria conducted a community-based study via door-to-door survey in a small town with stable population of about 20000 people. Potential cases identified via screening questionnaire and basic evaluation, were investigated further by a neurologist at the local hospital (details not supplied by the authors). They found a crude prevalence ratio of 15/100000 for MND (Osuntokun et al., 1987). The study by Tekle-Haimanot et al was very similar, yet conducted in Ethiopia. They found an MND prevalence of 5/100000. All cases had onset before age 42 years (Tekle-Haimanot et al., 1990).

In conclusion, the data from primary African MND studies is sparse, with most information coming from retrospective audits. There is therefore great need for prospective observational studies in Africa.

#### *Studies on MND in populations with multiple racial groups, including mixed African genetic ancestry*

Eleven original studies looking at MND in mixed populations were found via the search strategy: five European (Tomik et al., 2000, Rojas-Garcia et al., 2012, Gouveia and De Carvalho, 2007, Johnston et al., 2006, Elian and Dean, 1993), four from the USA (Rechtman et al., 2015, Noonan et al., 2005, Kazamel et al., 2013, Gundogdu et al., 2014) and two from other regions (Zaldivar et al., 2009, Drory and Artmonov, 2007). In addition two reviews were found which discussed variation in MND in different populations (Chancellor and Warlow, 1992, Cronin et al., 2007), but neither had additional information about African MND.

Chancellor examined the epidemiology of adult onset MND in populations "across the world". Although he did not consider African populations, he did note marked variability in incidence in different population groups and considered genetic and environmental factors as contributory (Chancellor and Warlow, 1992). Cronin et al reviewed several MND epidemiological studies. The African studies included have already been examined here in their original form (Cronin et al., 2007).

Of the 11 original studies, five were found to render more information and will be considered in more detail. The earliest of these, conducted by Tomik et al in 2000, was a retrospective case control study from the King's Motor Neuron Disease Care and Research Centre, London. It looked at phenotypic differences between black and white patients with MND. It included 15 black patients with West Africa/Caribbean ancestry with three age- and sex-matched white MND patients for each black case. These investigators found a four times higher chance in the black African group of presenting with the flail arm variant (OR 4.33,  $p=0.05$ ). Other potentially interesting findings were no familial cases in the black African group (compared with four in the white group), and one African case with sensory symptoms without signs (Tomik et al., 2000).

Drory et al. published a study from Israel in 2007, looking at the presentation of MND in various subpopulations with the same living environment but differing genetic ancestry. The study was a retrospective analysis of 374 sporadic ALS cases (ancestry: 211 Central and Eastern European, 53 North African, 43 Oriental, 19 Balkan, 9 Arab and others). The North African group was 57% male, 32% had initial bulbar presentation and mean age at onset was 52.4 years. The main differences found between the groups were significantly younger age at onset in the African and Arab groups, and a shorter duration of disease (relative to age) in the African group (Drory and Artmonov, 2007).

Kazamel et al in 2013, retrospectively assessed differences between black and white MND patients attending a clinic in Alabama, USA. They reviewed records of 207 patients (147 white and 60 black African-American) with a similar racial proportion to that found in the local population. The black group was significantly younger at onset of disease than the white group (55 years versus 61 years,  $p = 0.011$ ), but there was no difference with respect to survival or clinical features. In both groups longer survival was linked to younger age (Kazamel et al., 2013).

A study by Gundogdu et al. in Arkansas, USA in 2014 focused on a death certificate review to analyse the number of deaths due to MND by race. This information combined with clinic diagnostic rate statistics, showed a lower than expected rate of MND in the African American group, as compared to the local population racial demographics. The average age of onset was again significantly lower in the black group, and this group was found to be less functionally able at presentation. There were no familial cases among the black African group, compared with 8% of white cases (Gundogdu et al., 2014).

Rechtman et al conducted an epidemiological study of MND cases in different geographic locations across the US; 5883 cases were collected over a 3 year period via active case surveillance by neurologists (75% white, 9% black African American, 4% Asian, 12% other race). Using the population demographics for the studied areas, crude average annual incidence rates for ALS were calculated, with breakdown by racial group: 1.79/100000person-years (PY) for whites, 0.8/100 000PY for blacks, and 0.76/100 000PY for Asians. In addition to this lower incidence rate, the black group were more likely to be younger than the other groups at disease onset (Rechtman et al., 2015).

Of the remaining studies, four rendered epidemiological data, largely relative rates of MND across racial groups. Noonan et al studied death registry data and compared variation in MND by demographic variables. African-Americans and Hispanics had considerably lower rates of mortality due to MND than Caucasians (Noonan et al., 2005). Zaldivar et al from Cuba found a lower mortality rate due to MND in a mixed race group, when compared to white and black Cubans (Zaldivar et al., 2009). Johnston et al studied MND incidence rates by racial group in various parts of London. It was a small study, but suggested a lower proportion of black African cases than would be expected for the population size (Johnston et al., 2006) but another study performed later by Rojas-Garcia et al also from London, found no significant difference in the incidence of MND by racial group (Rojas-Garcia et al., 2012).

Gouveia et al from Portugal reviewed a case series of 27 young onset sporadic MND cases (onset less than 25 years): nine with black African ancestry, four Caucasian and 14 of uncertain race. These cases all had longer survival than typical MND, in addition most were male and presented with symmetrical limb involvement (either upper or lower). Some of the cases underwent post-mortem and unusual basophilic inclusions were found within motor neurons. It is suggested that this presentation may represent a subtype of MND (Gouveia and De Carvalho 2007). Although this is a small sample, there is a high representation of black African ancestry patients with this unusual phenotype, again emphasising earlier age of onset within this group.

The study by Elian and Dean was not considered further as it used only study participant name as a method of racial classification of these subjects (Elian and Dean, 1993).

Johnston et al. highlighted an important point regarding studies of MND in mixed genetic ancestry populations, namely that numbers of cases can vary widely according to definitions and inclusion criteria. These factors are not standardised across studies, making comparative analysis difficult (Johnston et al., 2006).

#### *What is known about MND-associated genes in people of black African genetic ancestry?*

There was very limited literature on MND genetics in African people. Four studies mentioned known MND genes and people of African genetic descent. The earliest study was that of Guerreiro et al from 2008. This group looked at the frequency of *TARDBP* mutations in sporadic ALS. 279 ALS cases (Caucasian) were examined, as well as 806 neurologically normal controls of European descent and a further 173 African control samples. Overall no cases had the mutation of interest but Africans were found to have greater genetic variation in *TARDBP*; single nucleotide polymorphisms (SNP) were more common (6.8%) than in Caucasian controls (1.1%) consistent with ancient origins of African populations (Guerreiro et al., 2008). This finding of increased SNP variants in African populations compared to European and Asians was also reported by Schuster et al (Schuster et al., 2010).

Kwiatkowski et al. first described the *FUS* mutation causing familial ALS in a family from Cape Verde Island, off the North African coast (Kwiatkowski et al., 2009). This *FUS* protein is a neuronal nuclear protein involved in DNA and RNA processing. In cases with a *FUS* mutation, the protein aggregated abnormally within the cytoplasm causing cellular dysfunction. This is a similar mechanism to what happens with *TARDBP* mutations.

In 2011 DeJesus-Hernandez described a case of sporadic ALS with a *VCP* mutation in a patient of African-American descent (DeJesus-Hernandez et al., 2011). This gene encodes for a protein with multiple intracellular roles including autosome-phagosome degradation. The mutation was an amino acid substitution or non-synonymous SNP, and was felt to have a disease-causing role in their patient. Other *VCP* mutations had previously been described in ALS (Johnson et al., 2010).

Garcia-Redondo et al published a paper looking at the frequency of the *C9orf72* repeat expansion in Spanish ALS cases and controls. Further controls from different racial backgrounds were included including European, African, Chinese and Japanese individuals. The expansion was found in 27.1% of the familial ALS cases and 3.2% of the sporadic cases. None of the controls had the expansion. In addition, the haplotype flanking the genetic alteration was shared amongst the cases. Amongst the controls, this expansion-associated haplotype was noted to occur relatively commonly in the European group (8.9%), and the African group (5.6%) but much less commonly in the Asian groups (1.6%). The authors suggested the relatively high frequency of the expansion-associated haplotype suggested it was ancient and that different mutation events could have occurred independently on a similar background (opposite of a more recent founder-effect). They suggest that this haplotype could be more prone to expansion mutations, such as the *C9orf72* expansion (García-Redondo et al., 2013).

No more specific genetic studies have been conducted in African populations with MND and specifically there are no genetics studies in Southern Africans.

## **Discussion**

On reviewing the available literature on MND as it occurs in Africans, it becomes apparent that little is really known. Although there are a few original studies, all are very small and most are very out dated. Study methods vary widely, and are not rigorously scientific in most instances. There is also potential overlap with other motor neuron disorders such as polio, konzo or spinal muscular atrophy (SMA), as some studies did not specifically exclude these disorders and these may account for the earlier age in onset and longer disease durations. There are no specific reports pertaining to the genetic investigation of MND in African patients.

In full consideration of the above, some points regarding MND in African patients may be concluded. Most of the studies reported younger age at onset of disease in people of African genetic ancestry. There also appeared to be a tendency to longer survival. The reasons for this are unclear, and may relate to mixed reporting of different types of motor neuron disorders, including for example SMA. There were a few reports suggesting reduced incidence of MND in African populations, but this was studied in indirect ways and could reflect ascertainment bias or poor access to resources. Rates of familial disease appeared lower than in Caucasian populations, but again this could be inaccurate given the small sample numbers and lack of clarity in how this was determined. Phenotypic differences were not widely examined. One study (Tomik et al., 2000) found an increased rate of flail arm variant in patients of African genetic ancestry, and another from Sudan (Abdulla et al., 1997) found bilateral upper limb involvement at presentation was most common. Bulbar presentations were also relatively common in two studies (Osuntokun et al., 1974, Cosnett et al., 1989). Two studies mentioned sensory symptoms without signs (Wall and Gelfand, 1972, Tomik et al., 2000), but again the numbers are extremely small and this could possibly reflect inaccurate diagnoses. In the absence of a definitive test for MND it is important that the diagnosis is reviewed in the face of unusual clinical findings. For example, a patient referred to Groote Schuur Hospital as a young-onset MND case was found to have severe motor neuropathy due to molecularly confirmed porphyria variegata (Albertyn et al., 2014). That said, more recently there have been reports of clinical, electrophysiological and pathological evidence of sensory nerve abnormalities in a minority of MND patients (Hammad et al., 2007, Nolano et al., 2016).

It is therefore still unclear whether MND occurs differently in people of African genetic ancestry. To be able to answer this question, more, larger-scale prospective clinical and epidemiological studies on MND are required from all over Africa.

Africa is a geographically vast area with distinct subpopulations, more broadly considered as Western, Eastern and Sub-Saharan Africa, but actually comprising more than 2000 different ethnic and language groups. Studies examining the mitochondrial and nuclear DNA from African populations indicate that Africa is the most genetically diverse region in the world (Tishkoff and Williams, 2002). Even within sub-Saharan Africa, a recent study showed that five individuals of Khoisan and Bantu origin, from South Africa and the greater Kalahari region, were more distinct from one another in terms of SNP than a person of European ancestry is from one of Asian ancestry

(Schuster et al., 2010). Therefore although it may be simplistic to group “Africans” into one group, the richness of the genetic ancestry of its peoples surely means a greater potential chance of uncovering new variants responsible for MND which may impact on the understanding of the molecular underpinnings of this disease.

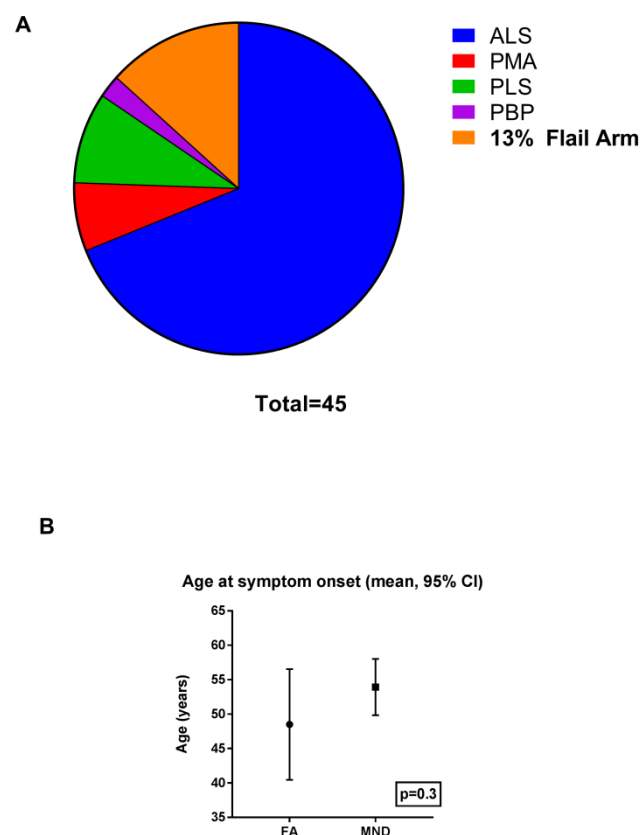


## 6.2 Flail arm variant of motor neuron disease:

### Clinical phenotype description of a case series from the Western Cape

During the time period March 2014 to September 2016, 45 patients attended the Groote Schuur Hospital MND clinic and were enrolled into the clinical research database and DNA repository. Of these, 15 were female and 30 male. Patients were asked to self-categorise according to South African census racial categories. 82% of clinic patients were of African genetic ancestry. Different MND subtypes were represented, including 69% amyotrophic lateral sclerosis, 7% progressive muscular atrophy, 9% primary lateral sclerosis, 2% progressive bulbar palsy and 13% flail arm variant (see figure 4, A). The mean age of onset in the flail arm variant group was compared to the rest of the MND subtypes as a group, but no significant difference was found (48 years vs 54 years,  $p=0.3$ ) (see figure 4, B).

The flail arm variant group will be described in further detail. For the purposes of this report flail-arm variant is defined as a form of lower motor neuron disease predominantly affecting bilateral proximal upper limbs, although possibly asymmetrically at onset. The clinical features remained restricted to this region for at least 12 months.



**Figure 4: Relative proportions of MND subtypes at GSH MND clinic during study period (A) and mean age at symptom onset in the FA variant group as compared to the rest of the subtypes (B).**

## Case 1

This 40 year old male of mixed African genetic ancestry (M/A) first presented a few months after symptom onset, with a complaint of bilateral shoulder weakness. He described an initial difficulty with lifting heavy objects above the level of his neck which progressed gradually over six months to not being able to lift up lighter weight objects. There were no lower limb, bulbar, visual or sphincter symptoms. He had previously been diagnosed with hypertension and was controlled on treatment. The patient had no significant family history.

On neurological examination, he was found to have normal higher functions and cranial nerves. In the motor examination, there was no neck weakness. The upper limbs showed normal tone on the right, but reduced tone on the left. In terms of reflexes, brachioradialis was reduced, biceps absent and triceps present. There was prominent proximal weakness bilaterally: shoulder abduction MRC grade 3 (out of 5) on the right, grade 2 on the left; elbows flexion/extension grade 3-4; distal grade 5. The lower limbs had normal power, tone and reflexes. Sensation and coordination were intact.

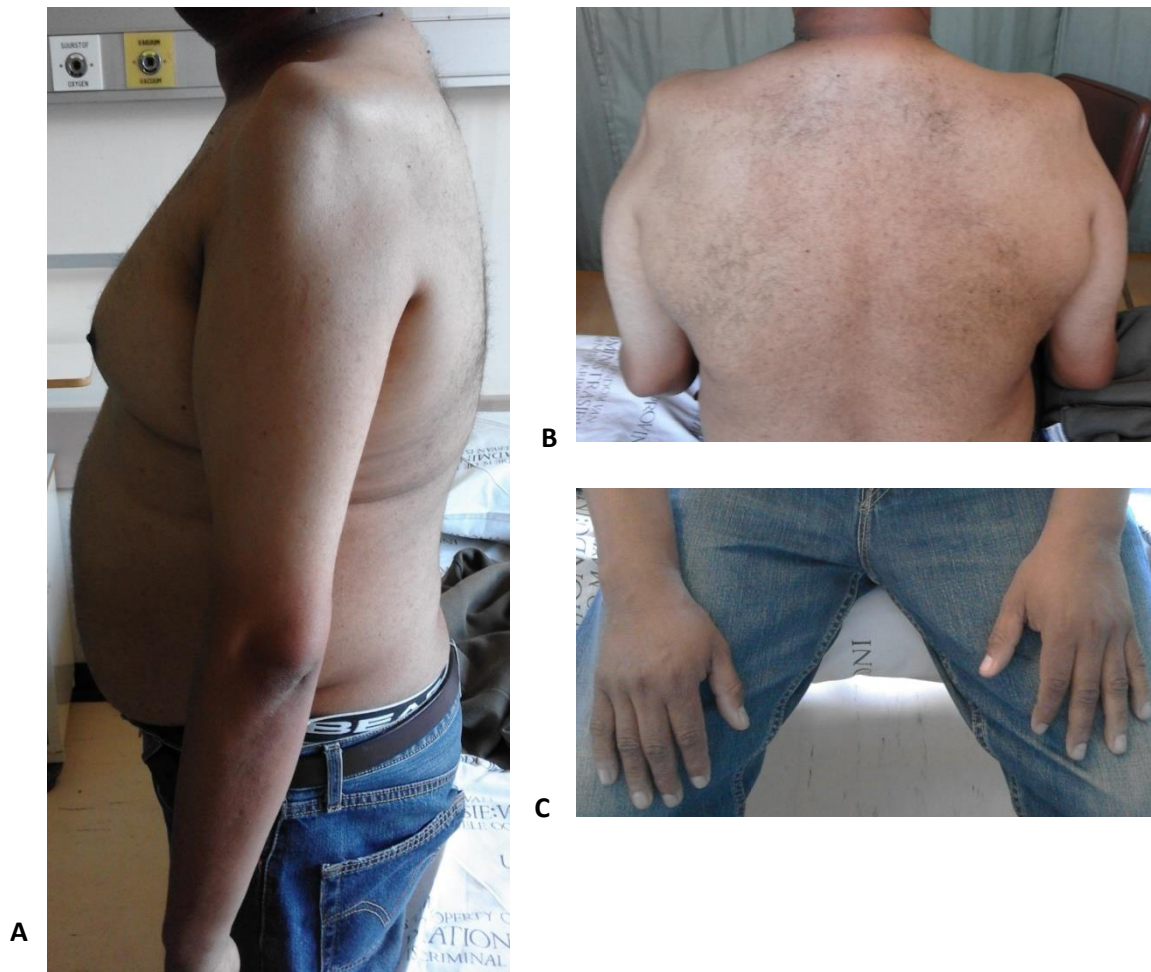
MRI scan of the brain and cervical spine were normal. Nerve conduction studies in the arms and legs were normal. Electromyography (EMG) of the left deltoid, biceps and first dorsal interosseus all showed large, polyphasic motor units with reduced recruitment. Only the deltoid and biceps showed spontaneous activity. Serum creatine phosphokinase was mildly elevated at 396 IU/L (normal < 180). Blood counts and other metabolic parameters were normal.

A muscle biopsy showed changes consistent with a longstanding neurogenic degeneration. Molecular genetic testing excluded a SMA mutation of the *SMN1* gene.

A year later he reported ongoing deterioration and had to be medically boarded from work. Fasciculations were noted in the deltoid and biceps muscles but power remained stable for almost 2 years although muscle wasting was increasingly prominent in the proximal arms.

Approximately 5 years later the patient began to complain of tiredness in his legs on walking. Examination showed wasting of the shoulder girdle muscles, and loss of all upper limb reflexes. Power was grade 2 for shoulder abduction bilaterally, 1-2 for elbow flexion, elbow extension and wrist flexion were 3, and wrist extension 4. Finger power was grade 4. There was also mild hip flexion weakness (grade 4) but the other leg muscles had full power. Tone and reflexes in the legs were normal.

The patient continues to be followed, now 7 years after the initial presentation. His proximal arm impairment (lower motor neuron) continues to dominate the presentation. Although he does have mild leg weakness, this is not functionally limiting. He still has no bulbar or respiratory symptoms.



**Figure 5: Photographs (with patient permission) of case 1, illustrating the pattern of marked proximal upper limb wasting (A, B), with sparing of more distal muscles (C).**

### Case 2

This 52 year male of M/A developed shoulder weakness starting on the left side, but with involvement of the right side within a few weeks. There was an initial fairly rapid deterioration over about 2 months with subsequent stabilisation. He had no difficulty with his hands or his lower limbs. There were also no bulbar or sphincter symptoms. The patient described paraesthesiae in his right hand 2<sup>nd</sup> and 3<sup>rd</sup> fingers. He was otherwise systemically well. He had no prior medical history but had suffered previous trauma – a screwdriver accident in 1982 leading to the loss of his right eye and a motor vehicle accident in 2009 with a minor whiplash injury to the neck.

On neurological examination, higher functions and cranial nerves examinations were normal except for the absent right eye. Motor examination showed marked wasting of the shoulder girdle muscles: both deltoids, pectoralis, supra- and infraspinatus muscles. Power in the arms ranged from MRC grade 2 for shoulder abduction to 4 at the elbows and 5 more distally. Supra- and infraspinatus power was graded at 3. Tone and reflexes in the upper limbs were normal. The lower limbs showed normal motor examination except for a reduced ankle reflex on the left side. Sensation was reduced over the middle 3 fingers of the right hand and the L5/S1 dermatome on the left side.

Further investigations showed MRI of the cervical spine and brachial plexus were normal without contrast enhancement. There was a mild disc prolapse at L5/S1. Nerve conduction studies showed a mildly prolonged distal latency in the right median sensory study consistent with carpal tunnel syndrome. EMG of the left deltoid and biceps showed spontaneous activity and chronic neurogenic changes in the motor units whereas trapezius and first dorsal interosseous were normal. Blood counts and metabolic parameters were normal. Serum creatine phosphokinase was 218 IU/L. Cerebrospinal fluid was normal.

Approximately a year later the arm weakness had deteriorated slightly. The patient had also developed neck flexion weakness finding it difficult to lift his head up off the bed. There was early swallow difficulty but forced vital capacity (FVC) was >80% of expected (4.2 litres). Motor examination showed fasciculations in the deltoid, biceps, brachioradialis and forearms bilaterally. Reflexes were absent in the upper limbs. Neck flexion power was MRC grade 3 and extension 4. Shoulder abduction and elbow flexion were severely weak, albeit asymmetrical. Distal arm power remained normal. Leg power was full.

Approximately 2.5 years after symptoms onset the patient noted mild hand weakness (grade 4) and later symptoms in his legs but with no functional limitation and no objective findings. About 3.5 years after symptom onset his dyspnoea on exertion increased, the FVC was 2.1 litres and there was mild dysarthria. Examination showed, the tongue was wasted and fasciculating. In the arms the wasting had progressed to involve the small muscles of the hands bilaterally. Power in the arms at the shoulders and elbows was 0, 2 at the wrists and 3-4 in the hands. The right leg had slightly increased tone with brisk knee reflexes and an upgoing plantar response on the right. Power was still normal in the legs. We were informed by the family that this patient died at home, approximately 4 years after symptom onset.

### Case 3

This patient 46 year M/A male presented with a 2 year history of gradual onset shoulder weakness. He was initially aware of left shoulder weakness and a few months later, he realised the right one was similarly involved. He had no lower limb or bulbar symptoms. He had no significant prior medical or family history. He did however have a heavy smoking history and had previously sustained tendon injuries to his hands.

On neurological examination he had normal higher functions and cranial nerve examinations. In the motor examination, he had significant wasting of his shoulder girdle muscles bilaterally with fasciculations, especially triceps, biceps, deltoid, pectoralis and supra- and infraspinatus, but more prominent on the left side. There was some milder wasting of the small hand muscles. Tone was reduced in the left upper limb with absent or reduced jerks, and normal tone and reflexes on the right. Power was reduced proximally with shoulder abduction at 3 on the left and 4 on the right, elbow flexion, wrist flexion and extension and finger extension were 4 bilaterally. Elbow extension and finger flexion showed full power. The lower limb motor examination was normal. Sensory examination was normal.

Nerve conduction studies showed reduced compound muscle action potential amplitudes in the studied upper and lower limb motor nerves (median, ulnar, tibial and peroneal) but normal proximal and distal conduction velocities. Electromyography of the deltoid, first dorsal interosseus, brachioradialis, tibialis anterior and masseter muscles showed active chronic denervation changes, and the rhomboidus major and orbicularis oris showed chronic neurogenic motor unit action potentials. Blood counts and metabolic parameters were normal. Cerebrospinal fluid was normal.

The patient was next seen 18 months later and he remained without bulbar or lower limb symptoms. His power had deteriorated slowly – predominantly in the proximal arms, which now showed a MRC grade of 2 for shoulder abduction bilaterally. His lower limb examination was still normal. His FVC was mildly reduced at 3 litres.

Four years after symptom onset he was still employed and working with his hands. He had however started to develop cramps in his lower limbs. His power grades remained the same but his upper limb reflexes had become brisk on the right side with spread, but normal tone.

#### Case 4

This 53 year male of indigenous African genetic ancestry presented with proximal left arm weakness, which had been slowly progressive over 2 years. He had no significant prior medical or family history. There were no sensory nor lower limb nor bulbar symptoms.

On general examination, he was considered to have gynaecomastia. His neurological examination of the higher functions and cranial nerves were normal. On motor examination of the upper limbs, despite unilateral symptoms, he had bilateral signs. He had asymmetrical wasting of the deltoids, biceps and triceps muscles (left > right). His tone was normal. Reflexes were normal in the right upper limb and absent in the left upper limb. Power was reduced bilaterally but asymmetrically: left proximal 1-2, distal 4+; right proximal 4, distal 5. His lower limb motor examination was normal.

Motor and sensory nerve conduction studies of the left arm and leg were normal. Electromyography of the left deltoid, trapezius and thoracic paraspinal muscles showed no spontaneous activity but large polyphasic units with normal recruitment – mild chronic neurogenic change. MRI of the patient's cervical spine showed no cause for the presentation. Blood counts and metabolic parameters were normal. Serum creatine phosphokinase was 358 IU/L. Cerebrospinal fluid was normal. Molecular genetic testing excluded an expanded CAG repeat in the androgen receptor gene responsible for X-linked bulbar-spinal muscular atrophy.

The patient was seen again 3 years later. Now 5 years into his illness, his arm weakness had worsened and continued to dominate the presentation. He had however also developed respiratory muscle weakness and a whispering dysphonia on standing. On examination, he had normal bulbar muscle appearance and function, although chest expansion was poor with a weak cough. There was mild neck flexion weakness of 4+. His upper limbs showed markedly reduced tone with absent reflexes. Power at the shoulder, elbow and wrist was 0-1. Finger flexion and extension scored a power grade of 2. Motor examination of the lower limbs was normal besides very mild hip flexion weakness of 4+/5.

Six years after symptom onset the patient deteriorated dramatically. He developed worsened weakness of his neck, trunk and lower limbs and was largely bedbound with dyspnoea on lying flat and inability to cough. On examination he had poor air entry in both lung bases with poor chest expansion. He was dyspnoeic at rest. His speech was dysphonic, but not dysarthric. His tongue had a normal appearance, and he had normal facial power. His jaw jerk was brisk. The neck was weak in flexion and extension at grade 3. Motor examination in the upper limbs showed reduced tone, absent reflexes and no power in any muscle group. The lower limb motor examination showed normal tone, brisk reflexes at the knees and full power except for hip flexion which scored a grade 4/5. The leg power had deteriorated further over the next few months to grade 3 at hip flexion and 4 to 4+ for the rest of the leg examination. The patient died at home approximately 7 years after symptom onset.

### Case 5

This 39 year male with M/A presented with mild weakness of his right shoulder which he had noted whilst weight lifting at gym for the past few months. He had no prior medical history, but had had previous injuries to both knees, sustained whilst playing soccer.

He was noted to have atrophy of his right upper arm muscles, fasciculations in the shoulder girdle muscles and mild scapular winging. His tone and reflexes were normal. Power in the right arm showed moderate weakness in the shoulder girdle muscles and triceps, mild weakness of the biceps and wrist extensors, and minimal hand weakness. The rest of the examination was unremarkable. MRI of the brachial plexus was normal. EMG was conducted which showed chronic neurogenic changes in the motor units of right C5 and T1 innervated muscles, as well as the mid thoracic paraspinals.

Two years after the initial symptom onset his right proximal arm weakness had become severe and the right arm reflexes were noted to be brisk. An MRI of his cervical spine showed mild degenerative disc changes but insufficient to explain his presentation. An MRI brain was normal. Four years later electrophysiological studies were repeated. Nerve conduction studies of the right arm were normal. EMG of multiple muscles in the right arm (deltoid, triceps, biceps and first dorsal interosseus) showed marked spontaneous activity and chronic neurogenic change of the motor units. EMG of the left deltoid also showed active and chronic neurogenic changes whereas only chronic changes were evident in the left first dorsal interosseous. EMG of right leg muscles (tibialis anterior and vastus lateralis) was normal.

Seven years post symptom onset, he reported to have experienced a gradual decline over the years in right arm power (predominantly proximally). He could no longer lift his arm above his head and could not grip normally. He had also noted fasciculations and was suffering with cramps in this arm. His other limbs were asymptomatic, and he had no bulbar, sphincter or respiratory symptoms.

He was found to be systemically well on examination and higher functions and cranial nerves were normal. On motor examination of the right arm, he had marked wasting and fasciculations around the right shoulder girdle, especially the rhomboids, deltoid, latissimus dorsi and pectoralis. Right arm tone was reduced, but reflexes were brisk. The Hoffman's was negative. Power was reduced both

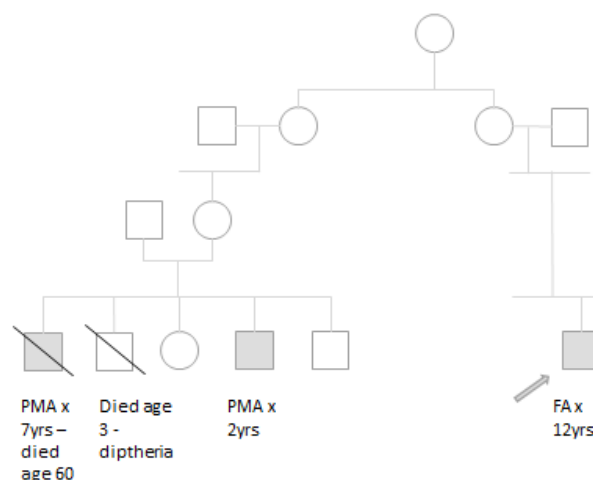
proximally (average MRC 3) and distally (average MRC 4). In the left arm he had subtle wasting of the left pectoralis, but all other parameters were normal. His lower limb motor examination was normal. Sensation was normal throughout.

EMG was repeated and confirmed the findings of two years previously; spontaneous activity and chronic neurogenic change in the right deltoid, triceps and adductor digiti minimi; left deltoid showed chronic neurogenic change. Right and left leg sample EMG was normal.

The patient returned for review approximately 12 years after symptom onset. He reported continued gradual decline in his right arm power. It was now markedly weak and wasted and had become functionally useless; he was using his left arm for all tasks. He had recently become aware that his head felt heavy, but had no difficulty in lifting it up from a supine position. He still had no symptoms in the left arm, either leg, bulbar or respiratory regions.

On examination, he was generally well. His forced vital capacity was 4.4 litres. Higher functions and cranial nerves were intact. Neck flexion power was mildly reduced at 4+/5, with normal extension power. The right shoulder girdle was markedly wasted with florid fasciculations, as were the forearm muscles but the hand was relatively spared. In addition, there was mild wasting of the left deltoid with fasciculations. Power was also markedly reduced (grades 0-2) throughout the right arm. The left arm showed mild finger weakness (grade 4) but was otherwise strong. Tone was reduced in the right arm with very brisk reflexes and a positive Hoffman's. Left arm tone and reflexes were normal. Sensation in the upper limbs was normal. Lower limb examination and gait were normal. A repeat MRI of the cervical spine again was non-contributory. A diagnosis of probable flail-arm variant of MND was reached despite the asymmetry of the signs.

Interestingly, at this time he became aware of a positive family history (see figure 6). Two male cousins had been diagnosed with a generalised lower motor neuron predominant MND (progressive muscular atrophy). The one case had died of his illness after 7 years. He was negative for the bulbospinal muscular atrophy and SMA genes.



**Figure 6: Partial genogram for patient 5 illustrating the significant family history**

### Case 6:

This 64 year M/A male presented with painless bilateral upper limb weakness, of slow progressive onset over the previous 3 years. Over the last year his hands had become weak as well, predominantly on the left side, which had limited his functional ability. There were no lower limb symptoms, nor respiratory nor bulbar symptoms.

This patient first presented to the orthopaedics department, about two years after symptom onset, and was found to have significant multilevel degenerative spine disease. MRI showed significant spinal stenosis secondary to disc-osteophyte complexes C3-C7, with associated bilateral foraminal narrowing C3-C7 and mild cord compression C4/5 but no associated cord signal change. A C3-C6 laminectomy was performed in early 2016, but despite marked interval improvement on repeat MR cervical spine imaging, the patients' signs and symptoms continued to worsen. In addition, no sensory changes were ever present. He was therefore referred to neurology.

On examination, the striking feature of the patient's presentation was marked bilateral, symmetrical upper limb weakness and wasting, with hypotonic arms that were almost functionally useless. Reflexes were present in the upper limbs. Power was reduced to MRC grade 2/5 at shoulder abduction and elbow flexion/extension. Wrist power was 4/5, as was hand power on the right side. Left hand power was reduced to 2-3/5. Fasciculations were seen throughout the upper limbs. Neck power was only slightly reduced on flexion (4+/5). The lower limbs showed mild bilateral spasticity and hyperreflexia with extensor plantar responses. In addition, fasciculations were seen in the quadriceps and tibialis anterior muscles. Power in the lower limbs was full. Bulbar examination was normal. Sensation was intact throughout the body. Despite not having any respiratory symptoms, chest expansion was found to be decreased and FVC was 1.4L.

Nerve conduction studies of the right peroneal and right median motor nerves showed mildly reduced amplitudes with normal conduction velocities. The right tibial and ulnar motor studies, including F responses were normal, as were upper and lower limb sensory studies. EMG of the right deltoid and first dorsal interosseus muscles showed active and chronic denervation changes. Similar findings were found in the right vastus lateralis, the thoracic paraspinals and the upper trapezius muscle. This study concluded widespread electrophysiological evidence of a motor neurogenic process.

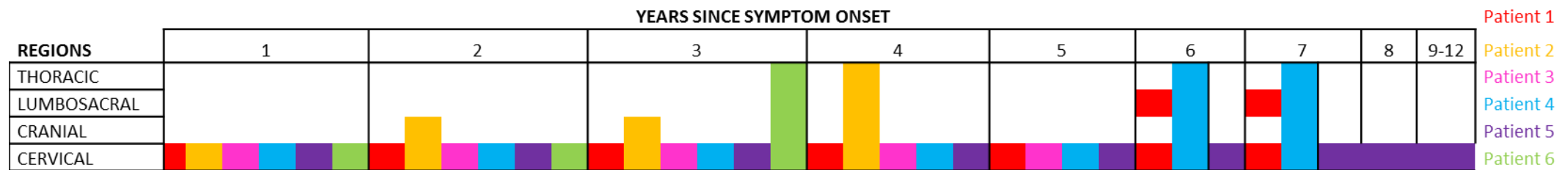
Although now three years into his illness and showing more widespread signs of motor neuron dysfunction, this patient's symptoms remain limited to his arms, and the proximal arm weakness and wasting continue to dominate the presentation. It is felt therefore that a diagnosis of probable flail arm variant can be made. Also to consider is that the patient did have co-existent degenerative spinal disease, which could account for some of the signs, including the lower limb UMN signs.



Case	Age at symptom onset	Duration of illness	Family history	Pattern of arm weakness (time from onset, to onset contralateral UL)	Pertinent clinical findings at first presentation	Initial diagnoses considered (prior to MND diagnosis)	Electromyography studies (year from symptom onset)	Appearance of UMN signs* or signs in other regions (years after symptom onset)
1	40 years (2009)	≥7years	Nil	B, P (0)	C5-C7 myotomal weakness and wasting (asymmetrical)	Spinal muscular atrophy; Limb girdle muscular dystrophy	C5/C6 acute/chronic; C8/T1 mild chronic neurogenic (1)	Mild LL weakness (5)
2	52 years (2012)	4years	Nil	U, P, (2mo)	C4-C7 myotomal weakness and wasting	Brachial plexopathy; Segmental CIDP	C5/C6 acute/chronic (1)	mild neck weakness & bulbar symptoms (1) Thoracic signs (3)
3	46 years (2011)	≥ 5years	Nil	U, P (6mo)	C5-6 myotomal weakness and wasting		C5-T1 (acute/chronic); V cranial; L4/5 chronic neurogenic (2)	UL UMN signs (3.5)
4	53 years (2011)	7years	Nil	U, P (2 years)	C5-T1 myotomal weakness and wasting	X-linked bulbar-spinal muscular atrophy	C3-5, T10 and cranial XI chronic neurogenic (2)	thoracic signs and neck weakness; UMN signs bulbar and LL (5)
5	39 years (2004)	≥12years	Maternal cousins with PMA	U, P (10years)	R side C4-C7 myotomal weakness and wasting	Brachial plexopathy; cervical myeloradiculopathy	R C5-C8, T10 chronic neurogenic (1) C5-8 bilateral acute/chronic (12)	UL UMN signs (2) Neck weakness (11)
6	61years (2013)	≥ 3years	Nil	B,P (0)	Prominent C4-C6 myotomal weakness and wasting (asymmetrical); milder LMN C8-T1	cervical myeloradiculopathy	C5-8, T10, L3, cranial XI acute/chronic (3)	U+L L UMN signs; thoracic signs (3 <sup>#</sup> )

**Table 4: Summary of flail arm variant case features**

(All cases were men. \*increased tone/reflexes, <sup>#</sup> seen for first time 3 years after symptom onset; B=bilateral, P=proximal, mo=months, UL =upper limb, LL=lower limb, R=right, PMA = progressive muscular atrophy)



**Figure 7: Graphical depiction of regional disease involvement over the years for each patient**

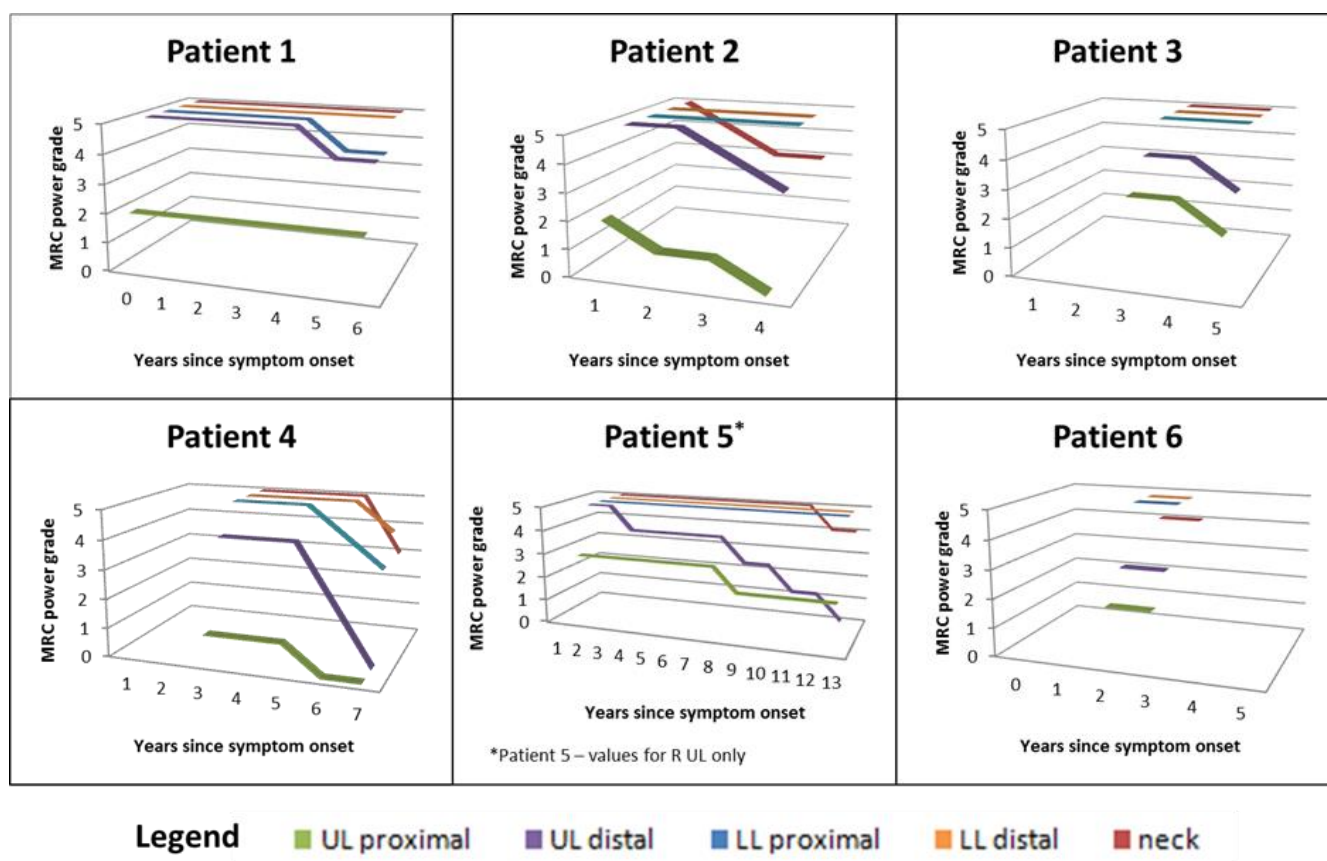


Figure 8: Graphs showing relative loss of power in body regions over time for each of the 6 patients

### Discussion of the case series

This collection of cases recruited from the Groote Schuur Hospital MND clinic between March 2014 and September 2016, illustrate in an African cohort, the previously described features of flail arm variant of motor neuron disease (Hu et al., 1998, Wijesekera et al., 2009, Katz et al., 1999, Hübers et al., 2015, Couratier et al., 2000, Talman et al., 2009). Although a seemingly clear phenotype, flail arm variant has to date been poorly characterised in terms of exact case definition, most likely in part due to the paucity of large published case series. The most widely used definition is that proposed by Wijesekera et al, which describes FA variant as a “lower motor neuron disorder of the upper limbs, characterized by progressive, predominantly proximal weakness and wasting with or without pathologic reflexes in the upper limbs, but excluding patients with hypertonia of the upper limbs, distal upper limb weakness or wasting without proximal involvement at presentation, and functionally significant weakness or wasting in lower limbs and bulbar musculature within 12 months of onset of upper limb symptoms.” (Wijesekera et al., 2009). We conformed to this definition of the FA variant in collecting this case series.

Within the GSH MND clinic, the FA variant cases represented 13% of the population during the study period. This is similar to other described cohorts, where the FA phenotype was found in 10% (Hu et al., 1998) and 6 and 11% (Wijesekera et al., 2009). In the series described by Katz et al., the FA variant cases made up only 2% of the overall cohort, but their inclusion criteria were more rigid (Katz

et al., 1999). Katz et al (Katz et al., 1999) are more restrictive in their definition, and define brachial amyotrophic diplegia (as they prefer to label the syndrome) as a pure lower motor neuron syndrome restricted to the upper limbs for at least 18 months, with the majority of patients having no signs outside of this region after a mean of 67 months. They propose that this group could perhaps rather be considered a variant of PMA than of ALS as a whole. Five of the cases in our series (cases 1,3,4,5) conform to this definition, with signs restricted to the upper limbs for at least 5 years from symptom onset.

Hübers et al reported that they considered the phenotype to be that of predominantly proximal, symmetrical involvement of the upper limbs at presentation (rather than symptom onset), and found according to this definition that 40% of their 42 cases had distal upper limb onset, 36% distal and proximal onset and only 24% purely proximal onset (Hübers et al., 2015). All of our cases had onset proximally in the upper limbs, but on first examination, four had milder distal weakness and wasting as well, although in three this was very subtle.

All of our cases were male which is in keeping with the previously noted male predominance (4-9:1) in all other described case series (Hu et al., 1998, Hübers et al., 2015, Katz et al., 1999, Wijesekera et al., 2009, Talman et al., 2009, Couratier et al., 2000, Tomik et al., 2000). This contrasts with the usual male to female ratio of 1.2:1 in typical ALS (Logroscino et al., 2008).

None of GSH FA patients were Caucasian – rather five of mixed African genetic ancestry and another of indigenous-Xhosa African ancestry. In the literature regarding patients with African genetic ancestry phenotypic differences were not widely examined. One study (Tomik et al., 2000) found an increased rate of flail arm variant in patients of African genetic ancestry, and another from Sudan (Abdulla et al., 1997) found bilateral upper limb involvement at presentation to be most common. Our cohort is too small to detect a potential increased rate of this variant in people of African genetic ancestry.

The FA variant patients in this series were mostly young on presentation (39-61 years, mean 48years). However the mean age of onset was not significantly different when compared to the rest of our MND cohort (48 vs 54 years,  $p=0.3$ ). Other FA variant cohorts also describe similar age of onset to ALS (Hu et al., 1998, Katz et al., 1999). The typically quoted mean age range of onset for ALS is 58-63 years (Kiernan et al., 2011, Logroscino et al., 2008).

Besides Katz et al, most of the published case series agree that upper motor neuron signs eventually develop in most patients over time. In this series four out of six patients developed upper motor signs with progression, but these were only detected  $\geq 3$  years after symptom onset. It is also generally agreed that spread to involve other spinal regions will eventually occur, although the predominant picture remains that of proximal upper limb weakness and wasting. In this series, in all patients, the proximal upper limb weakness and wasting continually dominated the presentation despite five out of six cases showing signs of spread of disease to other regions. There was marked variability in the time span over which this occurred (1-11 years). Of note, the case with the earliest spread had the shortest survival. This is in line with the observation by Wijesekera et al. who noted that the FA phenotype is associated with a significantly improved survival, which appears to be linked to time to spread to the second region of involvement (Wijesekera et al., 2009). This was also

shown in the series described by Katz et al. where the majority of the patients remained well with restricted LMN findings in the upper limbs after 67 months of symptomatic disease (Katz et al., 1999).

FA variant patients have been shown to have longer survival than typical ALS in most cohorts (Talman et al., 2009, Wijesekera et al., 2009, Katz et al., 1999), with a trend to longer survival in the cohort described by Hu et al. (Hu et al., 1998). One series from Japan however describes five patients with otherwise typically presenting FA variant, but with rapid development of respiratory compromise and death within 20 months from symptom onset (Kataoka et al., 2010). The authors suggest that genetic differences may be the cause. In the series described by Tomik et al., Caucasian FA patients are compared to FA patients with African genetic ancestry. The African ancestry patients displayed shorter survival than the Caucasian patients. We were unable to make direct comparisons between racial groups as our series of FA variant patients contained only mixed African genetic ancestries. However, although we did find variation in survival times (4 years to more than 12 years), no patients experienced a rapid progression as described in the Japanese cohort.

A case series from France (Couratier et al., 2000) showed the most common cause of death, as in other MND subtypes, to be respiratory failure. Interestingly though, in the FA patients, the majority of them had preserved independent ambulation despite respiratory failure. This is particularly interesting given the cervical origin of innervation of the diaphragm. Two of the patients in this series (cases 2 and 4) have died, presumably due to respiratory failure. Both cases were still independently ambulant at the time of death.

Case 1 and case 3 in this current series are perhaps the best prototypical flail arm variant examples, with complete restriction of clinical symptoms and signs to the upper limbs (with marked proximal predominance) for five and four years respectively, at which point there was only mild involvement of other regions. Case 4 remained similarly restricted for 5 years, but thereafter experienced a more dramatic deterioration. Case 2 does meet criteria for flail arm variant (Wijesekera et al., 2009), in that symptoms remained restricted to bilateral symmetrical proximal arm weakness and wasting for 15 months before spreading to other regions, and the arm involvement consistently dominated the presentation. He thereafter developed mild bulbar symptoms and later mild thoracic and lower limb symptoms too. One atypical feature in this patient is the mild neck weakness which became symptomatic early in the presentation (just 15 months post symptom onset). The neck muscles are supplied by nerves originating in the cervical plexus, which is also where the arm muscles are innervated from. This does therefore not necessarily imply that the pathology is more widespread than the cervical spinal segment earlier on. Early neck weakness is usually considered a poor prognostic sign (Nakamura et al., 2013). This patient remained ambulant with good speech and a relatively restricted disease pattern at three years into his illness, however despite this, he died about four years after symptom onset.

Case 5 is an atypical form of flail arm variant given the marked asymmetry of his presentation. Yet it has become apparent over time that the other arm is also involved. This patient otherwise displays features typical of FA variant, given the lower motor neuron onset in the proximal arm muscles (with only much later development of brisk reflexes) and restricted phenotype over many years. Monomelic amyotrophy (Hirayama et al., 1963) was considered as a diagnostic possibility, but the

patient is much older than the age range described for this condition. Monomelic amyotrophy also typically affects muscles innervated by C7-T1, only very rarely has fasciculations and is a fairly benign disorder, not progressing beyond a few years. Case 3 and 4 were also notably asymmetrical at onset, although did become more symmetrical with time. Hübers et al describe a large proportion (76%) of the cases from their series have asymmetrical onset which becomes symmetrical later on (Hübers et al., 2015).

The electrophysiological findings in cases 1 and 2 were restricted to the upper limbs muscles, and in fact only the proximal upper limb muscles in case 2. Cases 3, 4 and 6 showed more widespread abnormalities, on EMG in all cases and EMG and NCS in case 3 and 6. In these cases however the studies were done two to three years after initial symptom onset, whereas cases 1 and 2 had their studies done within a few months of symptom onset. This could illustrate a spread of pathology to other regions over time, but this is purely speculative. Interestingly case 5 had abnormal EMG of his mid-thoracic paraspinal muscles soon after symptom onset, although the most marked changes were in the symptomatic limb. Other FA case series which describe EMG findings (Sasaki, 2007, Yoon et al., 2014, Hu et al., 1998) also report more widespread chronic neurogenic change on EMG than is clinically apparent in terms of signs or symptoms.

One interesting aspect highlighted in this exercise was the varied differential diagnostic considerations in these patients. Only in case 3 was motor neuron disease diagnosed at the first presentation, although flail arm variant was only recognised later. This could indicate that flail arm variant is less widely known, but could also indicate an understandable reluctance to diagnose motor neuron disease in less than typical cases. This finding is not isolated to our unit. Hübers et al also reported an initial misdiagnosis rate of 55% for the FA variant cases (Hübers et al., 2015). They raise the point that this is detrimental, as many patients are being exposed to costly, ineffective and potentially harmful treatments – typically intravenous immunoglobulins for a misdiagnosis of multifocal motor neuropathy.

Case 6 is unusual in that he had definite co-morbid degenerative spine disease requiring decompressive surgery. It was only after this was corrected and the patient continued to deteriorate that an additional diagnosis of MND was made. It is not possible to determine how much the co-morbid condition influenced the presentation of MND. This situation is not unusual given how common degenerative spine disease is, and that the typical age of onset range overlaps with that of MND. A study from Japan found that 48% of ALS cases are complicated by co-morbid cervical spondylosis (Yamada et al., 2003). Another diagnostic consideration is cervical spondylotic amyotrophy, which is due to degenerative spine disease, presenting (usually unilaterally although bilaterally also described) with upper limb weakness and wasting affecting either proximal or distal muscles without sensory impairment or lower extremity dysfunction. MRI may show T2 hyperintensity within the cervical cord. There are two hypotheses about cause in this condition: either selective intradural ventral nerve root compression by posterolateral osteophytes, or vascular insufficiency resulting from dynamic cord compression. Autopsy studies have shown normal anterior horn cells, with selective intradural ventral nerve root compression. Cervical spondylotic amyotrophy is described to be clinically indistinguishable from FA MND in the early stages, but by three years, symptoms or electrophysiological changes should be present in other regions in the case of MND (Gebere-Michael et al., 2010, Jiang et al., 2011).

Genetic studies at our MND clinic are not routinely performed, however we have recently been able to test all our existing clinic patients for the *C9orf72* mutation. In people of European genetic ancestry, this mutation is known to 40% of familial ALS cases and 7% of sporadic cases (Majounie et al., 2012). In a much smaller sample of sporadic ALS cases in persons with African genetic ancestry, *C9orf72* mutation was found in 4% (Majounie et al., 2012). None of our cases tested positive for the mutation. (This could perhaps have been expected as the described mutations cause typical ALS; FA variant in association with *C9orf72* mutation has not been reported.) Further genetic studies are planned, including WES on two of these FA patients, including one family trio (as mentioned briefly in the methods section). The WES technique has recently been shown to be successful in detecting potential causative mutations in a familial FA variant: the study by Liu et al. describes a novel missense mutation in *hnRNPA1* in a large affected family (Liu et al., 2016). Other known MND genes with mutations previously linked to lower motor neuron presentations include *SOD1* and *FUS* (Ravits et al., 2013, Blair et al., 2010). A specific *TARDBP* mutation has been described for FA variant (Solski et al., 2012). These genes will be investigated further in our cohort at a later stage.

The two main limitations of this descriptive case series are the small size and its retrospective nature. Although the cases were identified amongst clinic attendees during a fixed time period, none of them were new presentations. This meant that information regarding the initial presentations, including examination and electrophysiological findings, were limited to that recorded in the patients' files. Additionally, the patients were differently investigated according to attending consultant opinions and varying differential diagnostic considerations. It did however enable the investigator to appreciate their progression over time. MND is a rare disorder, and the FA variant is only seen in a small proportion of cases. A larger case series would need to be collected over several years, ideally at multiple centres.

In conclusion, our case series illustrates the FA variant – a distinct MND phenotype with onset in the proximal upper limbs, predominantly lower motor neuron signs and a protracted course in most patients, which does seem to largely be linked to the extent of clinically apparent regional involvement. Katz et al. (Katz et al., 1999) felt that this variant should be limited to those patients with pure LMN involvement, restricted to the upper limbs for at least 18 months, but typically much longer. This subgroup does seem to have improved overall survival. The more inclusive definition proposed by Wijesekera et al. (Wijesekera et al., 2009) does however still have merit, as this phenotype does differ from other ALS cases in that ambulation remains spared until late, and the majority do experience a longer survival. We are limited by purely clinical definitions however, and perhaps MND pathology occurs as a spectrum rather than in discrete categories, making distinct classification difficult in some cases. We should however be aware that when counselling patients on prognosis, the greatly improved survival might only be relevant to those conforming to the stricter definition of FA variant as a persistently upper limb restricted, purely lower motor neuron variant.

With regards FA variant in patients of African genetic ancestry, with the current available information, we cannot yet comment on whether this variant occurs more commonly, but we can say that it occurs in a similar manner to that described for Caucasian populations. Larger studies from within Africa are required to address this question further.



## 7. Conclusions and recommendations

Considered in its entirety, this work has highlighted several important points regarding motor neuron disease, especially as it occurs in the African context.

More research is required on primary African populations to address the questions surrounding MND as it occurs in Africans, including phenotypic and genetic similarities or differences to other populations. A large prospective clinical study is currently underway at Groote Schuur and Tygerberg Hospitals to describe the incidence, clinical features and longitudinal course of motor neuron disease in the Western Cape.

Although controversy surrounding exact case definitions of the flail arm variant of MND remain, with further elucidation of underlying genetic and pathogenic mechanisms, the reasons for the phenotype and its prolonged survival may become clearer.

The genetics of motor neuron disease is still incompletely understood, but is currently a popular research topic, especially since the advent of next generation sequencing. Given the complexities involved, international collaboration with pooling of results is required to make the most of current knowledge and enable future progression. Further genetic studies are planned at Groote Schuur Hospital. We are currently building a DNA repository of all patients attending the MND clinic, with associated clinical findings, which will greatly assist in further genetic studies in our African MND population.



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